REMARKS

The Office Action

Claims 30-47, 50-51, 53-62, 64-72, 74, and 76-78 are pending in this application. Claims 32, 35, 38-42, and 45 are withdrawn from consideration. Claims 50 and 51 are objected to under 37 C.F.R. § 1.75(c), and claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. By this reply, Applicants amend claims 30 and 51, cancel claims 32, 35, 38-42, 45, 50, 74, and 76-78, and address each of the Examiner's rejections.

Objections under 37 C.F.R. § 1.75(c)

Claims 50 and 51 are objected to under 37 C.F.R. § 1.75(c) for improper format for failing to further limit the subject matter of a previous claim. The Examiner states that "Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form...[because c]laims 50-51 are...broader than the claims from which they depend" (Office Action, p. 2). Applicants have canceled claim 50 and have amended claim 51 to recite that the "polypeptide region has 100% sequence identity to the corresponding region of amino acid residues 1-185 of SEQ ID NO:2." In view of the amendment, claim 51 is now narrower than independent claim 30 from which it depends. This objection can now be withdrawn.

Rejections Under 35 U.S.C. § 112, first paragraph

Enablement

Claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 are rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner states that the specification is not enabling for:

> a method of lowering cholesterol in any mammal without inducing hypertriglyceridemia by intravascular administering to said mammal any vector comprising a nucleic acid molecule, including a recombinant adenovirus containing a nucleic acid encoding a polypeptide having fewer than 299 amino acids, wherein said polypeptide comprises a region of at least 150 amino acids having at least 90% sequence identity to the corresponding region of amino acid residues 1-185 of SEQ ID NO:2. (Office Action, p. 3; emphasis in original).

Applicants respectfully disagree that claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 lack enablement. Applicants have amended independent claim 30 to recite that the vector is "an *expression* vector" and that the nucleic acid does not encode amino acids 260-299 of SEQ ID NO:2. Applicants believe that the scope of claim 30, as presently amended, is enabled by the specification. Applicants address each of the bases for the present rejection in light of the present amendment to claim 30, and ask the Examiner to reconsider his position with respect to the enablement of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78.

Claim Breadth

The Examiner states that claim 30 is overbroad in that it encompasses treating "any mammal (e.g., a mammal lacking an endogenous normally functioning apoE gene, a mammal lacking an endogenous normally functioning LDL receptor or a mammal having...[a] lipid

disorder" (Office Action, p. 4). Applicants point out that the basis for the present invention is the discovery of a **biological role** for the N-terminal region of apoE (amino acids 1-259) in lowering cholesterol without inducing triglyceridemia. The N-terminal region of apoE mediates this activity by sequestering serum cholesterol in lipoprotein particles, which are eliminated by binding to LDL receptors, by binding to LRP, or by binding to heparin sulfate proteoglycans present on the cell surface (see, e.g., page 20, lines 5-20, of the specification). Therefore, when a polypeptide having a region corresponding to, or having biological activity equivalent to, the N-terminal region of apoE (i.e., excluding the C-terminal amino acids 260-299; e.g., a polypeptide having a region of at least 150 amino acids with at least 90% sequence identity to the corresponding region of amino acid residues 1-185 of SEQ ID NO:2) is expressed in a subject, the polypeptide will reduce the serum cholesterol level in the subject without inducing triglyceridemia, regardless of whether the subject lacks an endogenous, normally functioning apoE, lacks an endogenous, normally functioning LDL receptor (LDLR), or has a lipid disorder. Moreover, Applicants have demonstrated this biological activity in an accepted mouse model which, as is confirmed by Dr. Zannis in the accompanying Declaration (see, e.g., ¶¶ 4-6 of the Declaration of Dr. Zannis), is predictive of the biological activity of the truncated apoE polypeptide in a human. Thus, the scope of present claim 30, and claims dependent therefrom, is fully enabled.

Applicants have also amended claim 30 to recite that the vector is "an *expression* vector." This amendment clarifies that the method of present claim 30, and claims dependent therefrom, excludes delivery of the apoE polypeptide by "carriers such as cells administered by bone marrow transplantation" (Office Action, p. 4). As is clearly taught in the specification (see, e.g.,

page 11, lines 1-5), and as is known by one skilled in the art, an expression vector is "[a] vector, such as a plasmid, yeast, or animal virus genome, used to introduce foreign genetic material into a host cell in order to replicate and amplify the foreign DNA sequences as a recombinant molecule" (see, e.g., The American Heritage® Stedman's Medical Dictionary, 2nd Edition, 2004, Houghton Mifflin Company). Because expression vectors, and their use in promoting the expression of polypeptides in host cells, are well known in the art, the scope of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 is fully enabled with respect to this element as well.

Finally, the Examiner states that the scope of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 is not enabled with respect to "a nucleic acid encoding a polypeptide having fewer than 299 amino acids, wherein said polypeptide comprises a region of at least 150 amino acids having at least 90% sequence identity to the corresponding region of amino acid residues 1-185 of SEQ ID NO:2" (Office Action, p. 3; emphasis omitted). Applicants have amended independent claim 30 so that it now recites a nucleic acid that encodes a polypeptide that includes a region of at least 150 amino acids having at least 90% sequence identity to the corresponding region of amino acid residues 1-185 of SEQ ID NO:2, but which does not encode amino acids 260-299 of SEQ ID NO:2. The claims, as presently amended, clearly delimit the bounds of the nucleic acid molecule and the polypeptide encoded thereby. Specifically, the present claims exclude apoE polypeptides having amino acids 260-299 of SEQ ID NO: 2, which are the C-terminal residues of apoE involved in inducing hypertriglyceridemia. In addition to this structural limitation, the claims also clearly include a functional limitation (i.e., the ability of the polypeptide, when expressed in a patient, to lower the total serum cholesterol

level without inducing hypertriglyceridemia. Based on the structural and functional limitations recited in present claim 30, the scope of the present claims is commensurate with, and fully enabled by, the specification.

General Unpredictability in the Art

The Examiner maintains his reliance on Romano et al. (Stem Cells 18:19-39, 2000), Kawashiri et al. (Curr. Control Trials Cardiovasc. Med. 1:120-127, 2000), and Kypreos et al. (Biochemistry 40:6027-6035, 2001) to support a conclusion that lowering total serum cholesterol level in a patient without inducing hypertriglyceridemia by using gene therapy techniques is neither routine nor predictable (Office Action, p. 6). The Examiner also continues to rely on Tsukamato et al., Kayshap et al., Yoshida et al., and Athanasopoulos et al. to support a conclusion that it is unpredictable to use apoE proteins to maintain cholesterol and triglyceride homeostasis *in vivo* (Office Action, p. 7). Applicants respectfully disagree that the cited references demonstrate that present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 are not enabled.

M.P.E.P. § 2164.01 states that enablement of an invention, under 35 U.S.C. § 112, first paragraph, "requires a determination of whether the disclosure of the invention, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention." In addition, as was held by the court in *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991), an Applicant is not required to disclose every species even in an unpredictable art. The court stated:

> patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art...However, there must be sufficient disclosure either through illustrative example or

> terminology...to teach those of ordinary skill how to make and how to use the invention as broadly as it is claimed.

Applicants have plainly satisfied this requirement.

Applicants specification teaches how to make and use the invention and provides illustrative examples clearly demonstrating that the method of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 works successfully and predictably. As is discussed above, the invention is premised on Applicants' discovery of a biological role for the N-terminal region of apoE (amino acids 1-259) in lowering cholesterol without inducing triglyceridemia. To clarify this aspect of the invention, Applicants have amended claim 30 to require expression of an apoE polypeptide lacking the region encoding amino acids 260-299, which is the region responsible for inducing triglyceridemia. Applicants tested and verified their discovery by expressing an apoE polypeptide lacking amino acids 260-299 in a mouse model, which is an accepted model for predicting success in humans (see, e.g., ¶¶ 4-6 of the Declaration of Dr. Zannis). The specification clearly demonstrates that administration of an adenoviral vector resulted in successful infection of cells by the adenoviral vector and expression of the apoE polypeptide lacking the C-terminal amino acids 260-299 in those cells (see, e.g., pages 17-20 and Figs. 8-12 and 16). The specification further provides clear data demonstrating that, following infection, expression of the apoE polypeptide lacking amino acids 260-299 resulted in lower cholesterol levels in the infected mice without causing hypertriglyceridemia (see, e.g., page 29, line 23, through page 36, line 17, and, in particular, Figs. 8A and 8B). Thus, Applicants clearly demonstrate successful infection using the adenoviral vector, successful expression of an apoE lacking the C-terminal amino acids 260-299 following infection, and successful reduction in serum cholesterol levels without induction of hypertriglyceridemia. Applicants submit that the

illustrative examples provided in the specification are more than sufficient to demonstrate how to successfully and predictably make and use the invention. Therefore, the enablement requirement is satisfied.

Furthermore, as was asserted in the prior Reply to Office Action, filed on October 21, 2004, none of the references cited by the Examiner suggest that gene therapy does not work or is completely unpredictable; the references merely suggest that gene therapy has not been optimized sufficiently for certain (but not all) therapeutic ends. Applicants reiterate that, the cited references notwithstanding, the method of present claim 30, as presently amended, and claims dependent therefrom, is predictable, and the requirements for enablement under 35 U.S.C. § 112, first paragraph, are satisfied (see M.P.E.P. § 2164.01 and *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991). Thus, the rejection of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 for lack of enablement can now be withdrawn.

Direction of Guidance Provided by the Specification

The Examiner asserts that the guidance provided by the specification is insufficient to satisfy the enablement requirement. Specifically, the Examiner states:

> there is no indication...that the apoE-/- mouse model **is an acceptable model for lipid disorder**...Applicants do not provide any objective evidence indicating that ApoE-deficient mouse model is an acceptable model for any lipid disorder and that this mouse model mimics all the relevant conditions for human lipid disorders. (Office Action, p. 10; emphasis in original.)

The Examiner continues to rely on Kawashiri et al. and Orkin et al. to support this basis of the rejection. Applicants respectfully disagree that the specification fails to enable the present claims or that the cited references demonstrate that Applicants' specification fails to enable the present

claims.

As was explained in the previous Reply to Office Action, filed on October 21, 2004, neither Kawashiri et al. nor Orkin et al. disparages the use of apoE-/- mice as a model for lipid disorders. Kawashiri et al. and Orkin et al. clearly state that results in animal studies are often valuable, but that animal studies should be followed up with clinical trials in humans (see, e.g., Orkin et al., Executive Summary, paragraph bridging pages 1 and 2). Human testing is not required for enablement purposes to support claims of an *in vivo* utility. All that is required by the first paragraph of § 112 is "objective enablement" and, in a case in which the Patent Office questions the enablement of a claim (*In re Marzocchi*, 439 F.2d 220 (C.C.P.A. 1971)), evidence from sources other than human efficacy trails is acceptable. In this case, Applicants provide actual *in vivo* data demonstrating that the method of present claim 30, and claims dependent therefrom, can be practiced successfully and predictably (see, e.g., pages 17-20). As is discussed above, Applicants have tested and confirmed the method using a mouse model, which, as is attested to by Dr. Zannis, is an accepted model of the disease condition and is predictive of success in humans (see, e.g., ¶¶ 4-6 of the Declaration of Dr. Zannis). Thus, the Examiner is applying the findings of Kawashiri et al. and Orkin et al. far more broadly than is warranted.

In addition, Applicants note that the specification provides an amount of guidance that is more than sufficient to enable the skilled artisan to practice the method of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 successfully and predictably. This is demonstrated by the illustrative examples provided in Applicants' specification. Namely, the specification teaches the skilled artisan how to prepare viral vectors for delivery of the apoE polypeptide (see, e.g., page 20, line 23, through page 24, line 2, and page 39, lines 2-16). The specification also teaches several assays that the skilled artisan can use to identify an apoE

polypeptide for use in the method of present claim 30, and claims dependent therefrom. One assay involves analyzing the ability of the apoE polypeptide to decrease cholesterol levels without increasing triglyceride levels in an apoE -/- mouse that has been administered a viral vector encoding the apoE polypeptide (see, e.g., page 30, line 23, through page 32, line 3). The specification also provides guidance for identifying an apoE polypeptide for use in the invention by analyzing the plasma from adenovirus-infected apoE-deficient mice (see page 33, line 4, through page 34, line 20). ApoE polypeptides for use in the invention retain the cholesterol-lowering ability, as determined by clearance of cholesterol-rich lipoprotein remnants which float in the VLDL, and do not promote an increase in triglyceride production. The specification also provides an assay for determining whether the apoE polypeptide for use in the invention retains the ability to associate with pre-existing lipoprotein particles, which the specification teaches "is required for receptor-mediated lipoprotein clearance" (see page 28, line 14, through page 29, line 8).

Applicants also address the Examiner's reliance on Dijk et al., Linton et al., and Yoshida et al. to establish the non-enablement of the claims. None of the cited references demonstrate that Applicants' specification fails to enable the present claims. The Examiner, pointing to Dijk et al. and Linton et al., states:

> the instant claims encompasses a mammal that lacks an endogenous, normally functioning low density lipoprotein receptor (see dependent claim 46, for example). Both Dijk and Linton clearly demonstrated that over-expression of apoE in a lipoprotein receptor-deficient (LDLR-/-) mouse does not correct the hypercholesterolemia. Applicants failed to provide any guidance for a skilled artisan on how to lower the total serum cholesterol level without inducing hypertriglyceridemia in LDLR-/- mice, let alone for any LDLR-/- mammal as broadly claimed, and which is contradictory to the results reported by Dijk and Linton. Office Action, p. 10.

Applicants respectfully disagree that the teachings of Dijk et al. and Linton et al. are inconsistent with Applicants' data. Dijk et al. clearly indicates that, eight days following infection of apoE-/-, LDLR-/- mice with an adenoviral vector expressing full length apoE (Ad-APOE), serum cholesterol levels were significantly reduced relative to serum cholesterol levels in the control apoE-/-, LDLR-/- mouse (see Figure 1B on page 338). While Dijk et al. further indicates that the reduction of hypercholesterolemia in the apoE-/-, LDLR-/- mouse infected with Ad-APOE was associated with hypertriglyceridemia (see Fig. 1D on page 338), this result would be avoided using Applicants' method. As is discussed above, Applicants' method involves administering an apoE polypeptide that lacks the region responsible for causing triglyceridemia (i.e., amino acids 260-299 of apoE). Thus, practicing Applicants' method, in view of Dijk et al., would result in a reduction of serum cholesterol without a concomitant increase in triglyceridemia. Accordingly, the Examiner's reliance on Dijk et al. to prove non-enablement is misplaced.

Applicants also note that Linton et al. fails to prove the non-enablement of the present claims. Linton et al. demonstrates that, in the absence of LDLR expression, hepatic apoE expression is required for LRP-mediated remnant removal (see, e.g., page 1727). Thus, Linton et al. confirms that at least two mechanisms for cholesterol-enriched remnant lipoproteins exists: removal through the LDLR pathway and removal through the LRP pathway (see, e.g., the abstract). These pathways function independent of each other and serve as redundant mechanisms for cholesterol removal. Accordingly, when one pathway is disrupted, cholesterol removal along the other pathway continues to maintain cholesterol homeostasis. Therefore, the findings of Linton et al. do not negate the enablement of the present claims because even in the absence of the LDLR, apoE mediates the removal of cholesterol along the LRP pathway (see, e.g., page 1731). Moreover, as is discussed above, the findings of Dijk et al. confirm that

cholesterol removal can also be accomplished in an apoE-/-, LDLR-/- mouse. For these reasons, the method of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 (and in particular present claims 46) is not contradictory to either Dijk et al. or Linton et al. and is enabled to its full breadth.

With regard to Yoshida et al., the Examiner states:

> a vector also encompasses a cell expressing a recombinant human apoE because it comprises an exogenous nucleic acid encoding a human apoE and as written the claims do not encompass only apoE fragments having a C-terminal truncation. Thus, the breadth of the claims encompasses the method of Yoshida which reported negative results. Office Action, p. 11.

Applicants again note that claim 30 has been amended to recite "intravascularly administering to said mammal an *expression* vector" and to clarify that the apoE nucleic acid encoded by the expression vector "does not encode amino acids 260-299 of SEQ ID NO:2." Thus, claim 30 no longer reads on a method involving the administration of a cell or an apoE polypeptide having the C-terminal region identified as causing triglyceridemia, as was used by Yoshida et al. In any event, Yoshida et al. does not negate the enablement of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78. Yoshida et al. merely discloses that the expression of wild-type apoE3 in apoE-/- mice protects against the development of atherosclerotic lesions, while the expression of apoE2 and a variant apoE2, apoEcys142, do not provide this protection (see, e.g., the Yoshida abstract). If anything, Yoshida et al. clearly supports the enablement of the present claims because it confirms that an apoE polypeptide can be successfully and predictably expressed following administration using an adenoviral vector and that one skilled in the art can easily identify apoE polypeptides, using routine experimentation, that do not provide the required effect recited in present claim 30, and claims dependent therefrom (i.e., the ability to lower total serum cholesterol level without inducing

hypertriglyceridemia). Thus, Yoshida et al. does not support the rejection of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 for lack of enablement.

In view of the foregoing, Applicants respectfully submit that the full breadth of present claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-62, 64-72, 74, and 76-78 is enabled by the specification, such that one skilled in the art can successfully and predictably practice the claimed invention using no more than routine experimentation. Furthermore, the cited prior art does not negate and, in fact, supports the enablement of the present claims. For all of these reasons, Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. Enclosed is a petition to extend the period for replying for three months, to and including July 25, 2005, and a check for the fee required under 37 C.F.R. § 1.17(a). If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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